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(54) Title: PHARMACEUTICAL AEROSOL COMPOSITION AND APPLICATION THEREOF  
FOR TREATMENT AND PREVENTION OF VIRAL DISEASES

(57) Abstract:

The preparation contains, as an active substance, a proteinase inhibitor chosen from the group: aprotinin, its derivative or an aprotinin-like substance, in the form of an aqueous solution or solid micronized particles with the dimensions within the range 0.5-20  $\mu\text{m}$ . The aqueous solution contains the active substance in a quantity of 1,500-10,000 KIU/ml. The preparation is recommended to be used for the treatment and prophylaxis of preferably viral respiratory diseases.

**(57) Abstract:**

The preparation contains, as an active substance, a proteinase inhibitor selected from the group: aprotinin, its derivative or an aprotinin-like substance, in the form of an aqueous solution or solid micronized particles with the size ranging between 0.5 and 20 µm. The aqueous solution contains the active substance in the quantity of 1,500 – 10,000 KIU/ml.

The composition is proposed for use in treatment and prevention of predominantly viral respiratory diseases.

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## PHARMACEUTICAL AEROSOL COMPOSITION AND APPLICATION THEREOF FOR TREATMENT AND PREVENTION OF VIRAL DISEASES

### THE FIELD OF THE INVENTION

Present invention relates to a new pharmaceutical aerosol composition and its application for treatment and prevention of viral diseases.

### PRIOR ART

At present, a number of therapeutic substances are known, such as: amantadine (Douglas, R. G. Respiratory Diseases, 1979, pp. 413-425. In: "Antiviral Agents and Viral Diseases of Man" // Raven Press, NY, ed. By G. Galasso), ribavirin (Douglas, R. G. Ibidem, pp. 425-428), and biologically active polypeptides such as interferon (Scott et al., Brit. Med. J., v. 284, p. 1822 (1982)), which are capable of inhibiting an infectious process induced by orthomyxo--(influenza viruses) and paramyxo-viruses. Their therapeutic effect is due to the capacity to block reproduction of the above-mentioned viruses. Therapeutically, they are used in different medicinal forms: tablets, capsules, injection solutions and aerosols. The latter form is the most preferred. This is caused by the fact that during viral-bacterial respiratory diseases, the broncho-pulmonary epithelium is primarily affected, therefore the delivery of the active substance through the respiratory tract in the form of micronized particles of under 200  $\mu\text{m}$  in size would be the most effective. The application of the aerosol form of medicinal substances of the amantadine series is known. However, the above-mentioned therapeutic substances are active only against type A flu viruses, and inactive against type B flu viruses, paramyxoviruses and many other viruses, i.e., demonstrate a selective therapeutic effect.

There were also attempts to create antiviral medicinal drugs based on aprotinins –inhibitors of proteinases (Zhironov O. P., J. Med. Virol., 1987, 21, p.161-167). Aprotinins are natural low molecular polypeptides inhibiting a wide range of proteinases. The following officinal aprotinin preparations are known: Gordox (Gedeon Richter), Contrycal (Germed), Trasylol (Bayer AG), Antagosan (Behring).

The antiviral and therapeutic effect (inhibition of pulmonary pathology, prevention of animal deaths) was achieved by parenteral (intraperitoneal) inoculation of aprotinin. A sufficiently high dose of aprotinin preparation constituted from 10,000 to 15,000 Kallikrein Inhibiting Units (KIU) (animal/day). When inoculated parenterally, aprotinin polypeptides are absorbed into the bloodstream, where they change their physico-chemical (charge, ionization degree, molecular weight, conformation structure) and functional characteristics by binding with various blood proteins. Such protein complexing may result in masking and inhibition of aprotinin activity and blocking of the passage of the complexes from the blood into the respiratory tract. As a result, the antiviral effect mechanism is transformed and the therapeutic effect is reduced.

### BRIEF DISCRIPTION OF THE INVENTION

The main purpose of the invention was to develop a pharmaceutical aerosol composition based on biologically active polypeptide in the form of an aqueous solution or solid microparticles, which would provide an enhanced antiviral and pathogenetic effect during and

prevention in humans or animals suffering from respiratory tract diseases, when used in the reduced effective therapeutic dose.

This task is solved by the fact that the pharmaceutical aerosol composition of the invention contains, as an active substance, a proteinase inhibitor from the aprotinin group dissolved in water in the amounts from 1,500 to 10,000 KIU/ml of solution.

In order to improve stability of the aerosol and enhance its adsorption in the respiratory tracts, it is advisable that the composition additionally includes a surfactant, preferably Twin-80 or glycerol. The proposed composition is applied predominantly to affect different parts of the respiratory tract.

The aerosol, consisting of microparticles of aprotinin with different humidity, is prepared by atomization of an aqueous solution in the flow of air or a propellant by means of an atomizer providing a proper size of the particles depending on the localization site of the center of infection.

This task is also solved by the fact that the pharmaceutical aerosol composition of the invention contains, as an active substance, solid micronized particles of a proteinase inhibitor selected from the group consisting of aprotinin, its derivative and an aprotinin-like substance, the average size of which ranges from 0.5 to 20  $\mu\text{m}$ .

The dry powder or compressed powder aerosol of the composition according to the invention is obtained by means of powder inhalator or an aerosol device with a dosing device.

The powder aerosol with such particle size demonstrates high efficacy in treatment of viral diseases by administration into the respiratory tract. It is advisable to combine the powder aerosol composition with various pharmaceutically acceptable ingredients: a vehicle and a surfactant which stabilize the aerosol and enhance its efficacy during inhalations or applications in the respiratory tract. A pharmaceutical vehicle helps enhancing adsorption of the active substance in certain parts of the respiratory tract as well as prolongation of the therapeutic effect of the active substance, which results in reduction of doses during treatment and prevention.

Various vegetable oils (caster, mint, eucalyptus) as well as glycerin and non-ionic detergents, such as Twin-80, may be used as surfactants. These surfactants prevent aggregation and improve dispersion of particles in the aerosol composition, enhance the adsorption of the active substance by the respiratory tract epithelium and improve the therapeutic effect of aerosol. During application of the powder composition of the invention, it is used along with a pharmaceutically acceptable liquefied gas propellant in an aerosol device with a dosing device.

The aerosol composition may additionally contain other preventive and therapeutic preparations-synergists: pentamidine, bronchodilators and antibiotics.

The aerosol, consisting of micronized particles of the composition of the invention, provides an enhanced therapeutic effect on humans or animals when administered through the nasopharynx and the respiratory tract.

The aerosol of aprotinin, its derivative or an aprotinin-like substance provides a stronger therapeutic and a noticeable preventive effect when used in doses 100 times lower, than those needed for parenteral injections. The highest efficacy of the aerosol was achieved during its action on the lungs and lower respiratory tract, when the average size of the micronized particles was within 0.5 to 4  $\mu\text{m}$ . By increasing the particle size to 20  $\mu\text{m}$ , the best effect of the aerosol was achieved on the nasopharynx and upper respiratory tract. The enhanced therapeutic function of aprotinins, their derivatives and aprotinin-like substances is likely to be due to structural transformations of the active substance molecule as well as changes in targets of the pathogenetic action of the active substance upon administration of the aerosol into the respiratory tract as compared with parenteral injections of an aqueous solution.

As a result of transforming to the aerosol form, the conformation and ionization of the aprotinin molecule changed, especially at the phase separation boundary (air-particle).

The structural transformations, induced by aerosolization are likely to result in emergence of new physiological mechanisms and qualitative enhancement of the therapeutic effect of aprotinin and aprotinin-like substances.

In our opinion, the application of aerosols of aprotinin, its derivative and an aprotinin-like substance is possible not only by inhalation but also by instillation onto the microbe- or virus-affected areas of the body and mucous membranes, such as skin integuments, mucous membranes of the eyes, nasopharynx, throat, and the gastro-intestinal tract.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Provided below are the embodiments of the proposed invention referencing the drawings, in which:

FIG. 1 shows the dispersion profile of aprotinin aerosol microparticles;

FIG. 2 shows the dynamics of body weight changes in mice infected with influenza virus and treated with aprotinin aerosol;

FIGS. 3a, b, c, d show the protective effect of aprotinin aerosol in mice infected with A/Aichi/2/68 (a, c), Sendai/960 (b) and B/HK/72 (d) viruses.

#### DETAILED DESCRIPTION OF THE INVENTION

An aerosol composition has significant advantages over other medicinal forms for treatment and prevention of viral respiratory diseases. The aerosol composition of this invention contains, as the active substance, an inhibitor of proteinases selected from the group consisting of aprotinin, its derivative or an aprotinin-like substance, which are biologically active polypeptides.

Aprotinins and aprotinin-like polypeptides may be obtained from organs of various animals as well as from snails, snake poisons and from silkworm larvae. Aprotinin derivatives are obtained by modification of individual amino acids and covalent binding with the different groupings. The resulting aprotinin derivatives, while retaining the main biological and anti-

proteinase properties, acquire higher stability and are free from a number of side effects during medical application (Gerbhard, W. et al. "Proteinase Inhibitors". Elsevier Science Publishers, v. 10, p. 375-388, 1986).

Polypeptides are extracted and purified in an aqueous medium to achieve a pharmaceutically acceptable quality. To obtain aprotinin, its derivative or an aprotinin-like substance in the form of solid micronized particles, the aqueous solution of these purified polypeptides is subject to lyophilization and crush-grinding. The particle size is controlled by the grinding pressure. An average size of the formed particles ranges from 0.5 to 20  $\mu\text{m}$  and predominantly, from 0.5 to 10  $\mu\text{m}$ . The particles of aprotinin, its derivatives and aprotinin-like particles with the size of 0.5 to 4  $\mu\text{m}$  ensure the delivery of the active substance to the middle and lower parts of the respiratory tract and those with the size of 4 to 20  $\mu\text{m}$  – to the nasopharynx and upper respiratory tract.

Aqueous solutions of said polypeptides and micronized solid particles thereof may be used for inhalations. It should be noted that unlike other known polypeptides, for example, interferon, the polypeptides used in the invention are highly stable in the solid state and in aqueous solutions. They also retain their functional activity upon aerosolization, which was determined by trypsin inhibition by aerosol.

At present, tested preparations of aprotinins are available, such as: Gordox (Gedeon Richter), Contrycal (Germed), Trastylol (Bayer AG), Antagosan (Behring), which may be used for aerosol preparation.

To prepare an aqueous solution of aprotinin, Gordox or Contrycal, and Antagosan were used, which were diluted in distilled water so as to contain between 1,500 and 10,000 Kallikrein Inhibiting Units (KIU) in 1 ml of the solution. These doses were established as a result of experimental selection in the series of dose-effect type tests.

To increase the aerosol stability, it is proposed to introduce into the aqueous solution a surfactant, for instance, Twin-80, triton X-100 in amounts of 0.01 to 5.0 % by weight, which are readily dissolved in water. These surfactants enlarge the active surface of aerosol particles of aprotinin, its derivatives or aprotinin-like substance, thus enhancing their adsorption in the respiratory tract and improving the protective effect of the aerosol. Additionally, the solution may contain glycerol as a surfactant in the amount of 0.05 to 10 % by weight.

In order to prepare aerosol from the aqueous solution of said polypeptides, a pneumatic system of the ejector type can be used. The aqueous solution of the polypeptide is dispersed by evaporating a liquid fluorocarbon propellant or by forces of a high-speed gas stream, for instance, compressed air. Also, to make aerosol, an ultrasonic generator of Musson-I type (Trade Mark No. 25-2012.075-89, Altay Instrument-making Plant, Barnaul, Russia) may be used.

The aerosol cloud formation occurs due to ultrasonic dispersion of the aqueous solution.

In the first embodiment, the resulting aerosol consisted predominantly of particles ranging from 0.5 to 3.8  $\mu\text{m}$  in size. The inhalation of such particles ensures and most adequate delivery of aprotinin or aprotinin-like substances to the middle and lower parts of the respiratory tract.

It should be noted that due to rapid drying of the particles in the pneumatic mode, the resulting aerosol cloud consists predominantly of dry particles (90%), and the water concentration in said aerosol is about 0.01 mg/ml.

Due to the lack of airflow in the ultrasonic mode, a process of partial condensation of the aerosol and reduction of the intensity of particle drying takes place, so that the resulting aerosol has the properties of a humid aerosol.

In this aerosol, the particle size varied from 0.1 to 100  $\mu\text{m}$ , predominantly from 3.5 to 7  $\mu\text{m}$ , and during inhalation the delivery of the active substance to the nasopharynx and upper parts of the respiratory tract was ensured. This differentiation of aerosol delivery allows to select one of the possible ways of preparation and application of the aerosol depending on the localization of the center of infection. A positive therapeutic effects was achieved by using both dry and humid types of aerosol, however, the dry aerosol was better tolerated.

In order to produce the aerosol from the solid micronized particles of aprotinin, its derivatives or aprotinin-like substance, atomization of dry particles may be used either by means of an ejector type device or by means of "Spinhaler" or "Rotahaler" type insufflators. Another option is to atomize micronized particles from the suspension with a liquefied propellant.

In the first embodiment, the active polypeptide particles may be used in combination with a vehicle. The introduction of a vehicle facilitates preparation and standardization of the active substance powder and helps achieving its more effective delivery to the upper parts of the respiratory tract (nasopharynx, pharynx, larynx, trachea). The vehicle must be a non-toxic material, which is chemically inert towards said polypeptide, does not cause irritation of the mucous membranes and is acceptable for inhalation. Inorganic salts, such as: calcium carbonate, monosaccharides, for example, lactose, arabinose; polysaccharides such as dextrin, dextran can be used as a carrier. It is preferable to use lactose in the amount exceeding the active ingredient by 2-9 times by weight. The particle size of the aerosol, and powder aerosol in particular, is the main control factor when administering them into the respiratory tract. Therefore, the vehicle particles must also be of a certain size, particularly, not exceeding 200  $\mu\text{m}$ . When the size is larger, the aerosol particles may irritate the respiratory tract tissues, which is unacceptable. It is desirable for the vehicle particle size to be between 30 and 80  $\mu\text{m}$ .

In the second embodiment, the dry micronized particles of aprotinin, its derivative or aprotinin-like substance are suspended in a liquefied gas aerosol propellant and applied as a hermetic aerosol with a dosing device (valve). The liquefied propellant is a gas at room temperature (20°C) and atmospheric pressure, i.e., it must have a boiling point below 20°C at atmospheric pressure. The liquid propellant must be non-toxic. Dimethyl ether, chlorides of lower alkyls, fluorinated or fluorochlorinated propellants of lower alkanes or mixtures thereof may be used as propellants. Examples of such propellants include dichlorofluoromethane ("propellant 12"), trichlorofluoromethane ("propellant 11"), monochlorodifluorometane ("Propellant 22"), 1,2-dichlorotetrafluoroethane ("propellant 114").

A compressed powder aerosol composition of the invention may also contain a surfactant improving the dissolution of the active substance in the propellant and subsequent formation of

the aerosol particles, as well as enhancing adsorption and alleviating the irritating effect of the aerosol on the respiratory epithelium.

A surfactant may be a liquid or a solid non-ionic surface-active substance. Preferably, it would be a liquid surfactant selected from the group containing glycerin, vegetable oils (mint, castor, eucalyptus), oleic or palmetic acids, used in the amount of up to 5 % by weight. The mandatory condition for the use of a surfactant is its compatibility with the propellant. The higher the solubility of the surfactant in a propellant, the higher its activity as a surface-active substance.

When the composition is in the form of a dry non-compressed powder, i.e. in capsules for inhalation, a single dose of utilized polypeptide may vary from 0.01 to 20.0 mg, preferably from 0.05 to 0.5 mg.

When the composition is in the form of a compressed aerosol composition, it is desirable that the valve of the aerosol container ensures atomization of the single doses from 0.025 to 0.25 ml, preferably from 0.05 to 0.1 ml.

Below are the specific embodiments of the proposed invention.

EXAMPLE 1. Preparation of aerosol from an aqueous solution of aprotinin and calculation of single doses for mice.

An aqueous solution containing 1,500-10,000 Kallikrein Inhibiting Units (KIU) of aprotinin (Gordox) in 1 ml ( $C_a$ ) was dispersed into a mouse incubation chamber using ejector type pneumatic generator equipped with the compressed air supply system. The aerosol formation from the aqueous drops of aprotinin was caused by the forces of the compressed air flow. The aerosol mixture was supplied to the chamber at a rate ( $V_c$ ) of 9 liter/min, and the intensity of dispersion of the aprotinin solution ( $Q_a$ ) was 0.1 ml/min. The aprotinin concentration in the aerosol being formed was 0.017 to 0.12 KIU/ml as calculated using a formula  $C_c = C_a \times Q_a / V_c$ . The main part of the experiments with mice was carried out using an aerosol of the concentration from 0.05 to 0.10 KIU/ml. The dose received by a single mouse was calculated using a formula: Guyton =  $C_c \times C_t \times P \times V \times t \times R$  (Guyton, A. C., Am. J. Physiol., 947, v. 150, p. 70-77), wherein  $C_c$  is a relative cumulative concentration of aprotinin in the chamber at the time  $t$ ;  $P$  is a mouse weight (6 – 10 g);  $V$  is a specific respiratory volume of mouse (1.2 ml/g/min);  $R$  is a coefficient of retention of aerosol particles in the mouse respiratory tract (0.75). Since the velocity of air withdrawn from the chamber was equal to that of aerosol supplied to the chamber, the  $C_t$  coefficient was assumed being equal to 1. The calculation yielded an average dose of aprotinin to be 50 to 150 KIU/mouse/day (from 5 to 15 KIU per 1 g of mouse body weight per day). 1 KIU corresponds approximately to 0.35 TIU and 0.14  $\mu$ g of aprotinin protein.

To improve the aerosol stability in the atmosphere and to enhance its adsorption by the respiratory epithelium, in a number of experiments surfactants were added to the solution: a non-ionic detergent (Twin-80 or Triton-X-100) and/or glycerin to final concentrations of 0.05 to 10 vol. % and from 0.01 to 5.0 vol. %, respectively.

FIG. 1 shows the dispersion profile of aprotinin aerosol particles fed into the chamber. The diameter of the particles and their weight content in the aerosol was recorded by Malvern-2200 laser controller.

The ordinate axis represents relative weight content of the particles of a given size in the aerosol, wt. %, and the abscissa axis represents the category of the particles by size ( $\mu\text{m}$ ): 1 (diameter from 0.5 to 3.8), 2 (3.8-6.5), 3 (6.5-8.5), 4 (8.5-10.5), 5 (10.5-13.0), 6 (13.0-16.7), 7 (16.7-25.0), 8 (25.1-35.0), 9 (35.1-42.0), 10 (42.1-53.5), 11 (53.5-90.5). It can be seen that 95% of the particles had a size of up to 3.8  $\mu\text{m}$  in size, and 5% - up to 6.5  $\mu\text{m}$ . The particles of such size dried rapidly in the air flow, and the concentration of water in such aerosol approached the air humidity (about 0.01 mg/ml).

#### EXAMPLE 2.

In order to prepare compressed powder aprotinin aerosol, a dry powder, manufactured by A WD Company (Germany) under the name of Contrycal, or a vacuum-lyophilized powder from the frozen aqueous aprotinin solution, produced by Gedeon Richter and Behring Companies, were used. The powder was dispersed in an air-jet grinder under different pressures varying from 5 to 120 psig, followed by mixing of fractions with different degrees of dispersion to yield a mixture of particles ranging from 0.5 to 20  $\mu\text{m}$  in size.

0.06 ml of eucalyptus oil were dissolved in 6 ml of the mixture consisting of 3 wt. parts of "propellant 114" and 1 wt. part of "propellant 12", followed by suspending in this solution of 50 mg of said aprotinin powder, 250 mg of lactose with particle sizes ranging from 30 to 100  $\mu\text{m}$ , and 0.05 ml of Twin-80. the suspension was used to fill a 6 ml aluminum container with the valve calibrated for dispersion of a single dose of 0.05 ml. By pressing the valve, a portion of aerosol was dispersed into the chamber, where mice were incubated (8-18 feedings during an hour of incubation). The aerosol dose was chosen to create the concentration of aprotinin aerosol in the chamber close to those in Example 1. In order to maintain this concentration in the exposure chamber, aerosol was fed every 3-6 min.

The therapeutic and preventive effects of aprotinin aerosol prepared as described in Example 1 were studied on mice.

Influenza viruses A/Aichi/2/68 (H3N2) (orthomyxovirus), B/Hong Kong/72 and paramyxovirus Sendai/960, which are pathogenic for mice, were replicated in 9-day-old chick embryos. In the studies of chemotherapeutic and prophylactic effect of aprotinin aerosol, 1-100 of 50% mice lethal doses (MLD50) of virus, administered to mice via intranasal injections or inhalation of virus aerosol, were used. As a result of infection, the animals developed viral bronchopneumonia later joined by airborne microbes (viral-bacterial pneumonia).

The experiments were carried out according to the following scheme:

Mice were infected with A/Aichi/2/68 or Sendai/960 virus in a dose of 50 MLD50 /mouse, or B/HK/72 virus in a dose of 1 MLD50 /mouse and then one group of the infected mice (20-30 animals per group) was exposed in the chamber to a gas-water mixture, and another – in the

chamber with the aprotinin aerosol for 30-60 min 2 to 4 times a day. The pathological changes in the lungs were evaluated on the 5<sup>th</sup> day of infection, when the intensity of inflammation and virus titres in the lungs were close to the maximum. For this purpose, the lungs were removed from 2 mice of each groups and examined histologically and visually. The control mice treated with inhalation of gas-water mixture without aprotinin exhibited total hemorrhagic inflammation, which involved practically all (100%) the lung tissue (viral-bacterial pneumonia). In the mice treated with aprotinin aerosol inhalations, the intensity of inflammation was less pronounced and the hemorrhagic inflammation involved about 30 to 50% of the lung area. A similar pattern of lung involvement was observed in histological and macroscopic examinations of mice infected with Sendai/960 and B/HK/72 viruses and treated using a similar scheme with the aprotinin aerosol.

After intraperitoneal inoculation of mice, infected with 5 MLD<sub>50</sub> of A/Aichi/2/68 or Sendai/960 virus, with Gordox (aprotinin preparation) in the dose of 10,000 to 15,000 KIU/mouse/day, 5 to 6 injections daily for 6 days, a stronger lung damage was observed involving 40-60% of the lung area based on visual evaluation. Thus, the aerosol dose of 50 to 150 KIU of aprotinin inhibited the development of lung pathology more effectively than did parenteral injections of 10,000 to 15,000 KIU of aprotinin solution.

One of the criteria of therapeutic effect was also the weight gain of the animals, which, as has been established, is a sign of their recovery. FIG. 2 presents the dynamics of changes in the body weight of mice infected with influenza A/Aichi/2/68 virus. Mice, 20-30 animals per group, were infected with a virus dose of 50 MLD<sub>50</sub> /mouse and treated in the chamber containing aprotinin aerosol using the standard method. In the course of infection, the body weights were measured and mean values (in g) were plotted on the ordinate axis versus the number of days after infection, plotted on the abscissa axis.

Curve 1 represents the weight of uninfected mice, curve 2 – the weight of infected mice treated with aprotinin, curve 3 – the weight of infected mice of control group treated with gas-water mixture without aprotinin.

As can be seen from FIG. 2, in healthy (uninfected) mice the weight gain was approximately 1 g/day. In the group of mice infected with a lethal dose of influenza A/Aichi/2/68 virus, the weight remained unchanged and then began to decrease, which was associated with the animals' deaths. In the group of the treated animals, during the first several days of infection, only a slight weight gain was seen, however, starting from day 5 after infection, the weight gain was about 1 g/day, indicating recovery of the animals. A similar pattern of weight changes in the control and aerosol-treated mice was observed in the animals infected with Sendai virus. Thus, the application of aprotinin aerosol for animals suffering from the respiratory tract infection caused a positive therapeutic effect revealed in rapid normalization of the body weight gain.

At the final stage of study, the protective effect of aprotinin aerosol was evaluated in the experiments with a lethal dose of influenza A/Aichi/2/68 and B/HK/72 viruses or Sendai/960 virus. One should bear in mind that influenza B/HK/72 virus is less lethal for mice than the other two viruses.

FIGS. 3a, b, c, d illustrate the protective effect of aprotinin aerosol in mice infected with said viruses. The mice were infected with A/Aichi/2/68 virus (a, c), Sendai/960 virus (b) and B/HK/72 virus (d) at a multiplicity of 1 (d) or 50 (a, b, c) MLD<sub>50</sub> /mouse, and then for 6.5 days were given a course of aprotinin aerosol inhalations (a, b, d) or intraperitoneal injections (c) based on the scheme described above. In the course of infection, the animal survival rate was recorded. A number of survived mice (cumulative percent) was plotted on the ordinate axis, and the number of days after infection was plotted on the abscissa axis.. Curve 1 relates to mice of the control group, infected but untreated, and curve 2 relates to mice, treated with aprotinin aerosol.

FIG. 3d shows that aprotinin aerosol has protected from death 50% of mice, infected with 1 MLD<sub>50</sub> of B/HK/72 virus. Since with the infecting doses of 1 to 10 MLD<sub>50</sub> of influenza A and Sendai viruses, both methods of treatment (parenteral injections and inhalations of aprotinin aerosol) gave approximately 90% to 100% protection, massive infecting doses were used for comparison of these methods of treatment: approximately 50 to 100 MLD<sub>50</sub> /mouse. As can be seen from FIG. 3 (a, b), in the group of untreated animals 100% lethality was observed within 5-7 days in influenza (panel a) and within 6-8 days in paramyxovirus infection (b). In the treated animals, deaths started from 1 to 4 days later than in the control mice, and the protective effect was about 40% to 50%. In a parallel group of mice treated by intraperitoneal injections of aprotinin (15,000 KIU/mouse/day, 5-6 injections daily for 7 days) the protective effect was 30% as can be seen in FIG. 3c. The results of this series of experiments provided final confirmation to the conclusion of high therapeutic efficacy of application of low doses of aprotinin aerosol.

To evaluate a preventive effect of aprotinin aerosol, the experiments were carried out in uninfected mice (10 animals) combined with two other mice, infected with influenza A/Aichi/2/68 virus at a multiplicity of about 0.1 MLD<sub>50</sub> /mouse. Such groups of 12 animals were exposed to the gas-water mixture and aprotinin aerosol for 6-7 days, 3-4 exposures daily for 30-60 min each. On day 8, mouse lungs were examined. In the control group that was given the mixture without aprotinin, the centers of inflammation were detected in the lungs and in a number of cases the virus could be detected in the lungs. In the animals treated with the aerosol, no virus or centers of inflammation could be detected. Thus, in this group, a preventive effect of aprotinin aerosol was observed demonstrating limited virus transmission from infected mice to healthy ones.

Additionally, in the experiments using an aqueous solution of aprotinin prepared according to Example 1, the effect of surfactants, in particular, Twin-80 and glycerol, on the therapeutic efficacy in mice with influenzal bronchopneumonia was studied, and the results are presented in the Table below.

No.	Aprotinin aqueous solution	Therapeutic effect of aerosol (% of protection of mice)
1	Without surfactant	48
2	0.3 vol. % Twin-80	60
3	0.2 vol. % glycerin	56

As can be seen from the Table, introduction of surfactants into the aerosol composition increased the protective effect of the aerosol by 10-20%.

The compressed powder aerosol, prepared as described in Example 2, was tested experimentally using infected mice, incubated in the exposure chamber as described above while using aqueous aerosol composition. In this series of the experiments, it was found that the protective effect of the dry aprotinin aerosol with particle size ranging from 0.5 to 100  $\mu\text{m}$  was about 40-60% for influenza A and Sendai viruses.

The initial clinical trials carried out during an influenza outbreak caused by influenza type H3N2 virus, have demonstrated the therapeutic efficacy of aprotinin aerosol (Gordox preparation) administered by inhalation through a face mask or head chamber to children with influenza. A reduction in antigen-carrier period was observed: in untreated patients, viral antigens could be detected in the nasopharynx for approximately 6 days, and in the patients treated with aprotinin aerosol it was reduced to 3 days. This points to a quicker elimination of the virus from the body. The application of aprotinin aerosol proved to reduce the persistence of the disease symptoms (running nose, coughing, catarrh of the upper respiratory tract) by 2-3 days and prevented the development of secondary complications.

## FIELDS OF APPLICATION

The pharmaceutical aerosol composition based on aprotinins their derivatives or aprotinin-like substances may find large-scale application in medicine and veterinary practice as a therapeutic and preventive drug against a large group of viruses (influenza, parainfluenza, pneumoviruses, measles, mumps, respiratory-syncytial virus, coronaviruses, rhinoviruses, adenoviruses), the causative agents of many respiratory tract diseases in humans and animals.

What is claimed is:

1. A pharmaceutical aerosol composition, comprising as an active substance a proteinase inhibitor from the group of aprotinins dissolved in water in the amount of 1,500 – 10,000 KIU per 1 ml of solution.
2. The pharmaceutical aerosol composition of claim 1, which additionally comprises a surfactant.
3. A pharmaceutical aerosol composition, comprising as an active substance solid micronized particles of a proteinase inhibitor selected from the group consisting of aprotinin, its derivative or aprotinin-like substances, the average size of which ranges between 0.5 and 20  $\mu\text{m}$ .
4. The pharmaceutical aerosol composition of claim 3, *wherein* the active substance is combined with a pharmaceutically acceptable vehicle.
5. The pharmaceutical aerosol composition of claim 3, which additionally contains pharmaceutically acceptable liquefied gas propellant.
6. The pharmaceutical aerosol composition of claim 4, which additionally contains pharmaceutically acceptable liquefied gas propellant.
7. The pharmaceutical aerosol composition of claim 5, which additionally comprises a surfactant.
8. The pharmaceutical aerosol composition of any of the claims 1 or 2 for use in treatment and prevention of viral diseases in humans and animals.
9. The pharmaceutical aerosol composition of any of the claims 3 – 7 for use in treatment and prevention of viral diseases in humans and animals.
10. The pharmaceutical aerosol composition of claim 3, which is effective when the active substance is delivered to the lungs and the lower part of the respiratory tract, *wherein* the average size of the micronized particles is 0.5 – 4  $\mu\text{m}$ .
11. The pharmaceutical aerosol composition of claim 3, which is effective when the active substance is delivered to the nasopharynx and the upper part of the respiratory tract, *wherein* the average size of the micronized particles is 4 – 100  $\mu\text{m}$ .

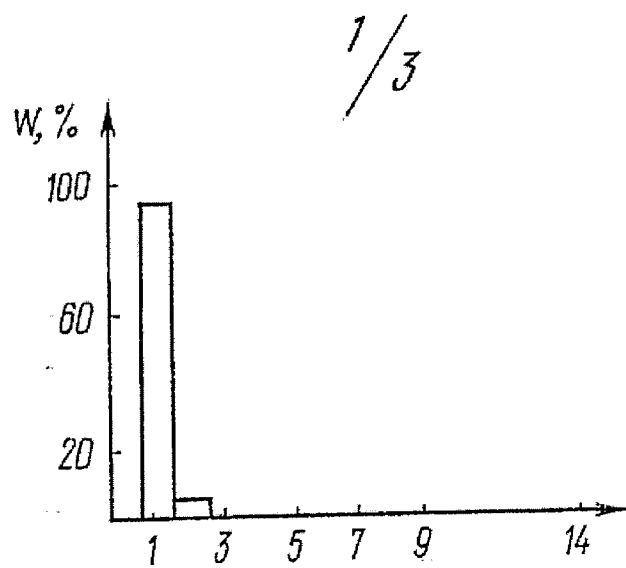


FIG.1

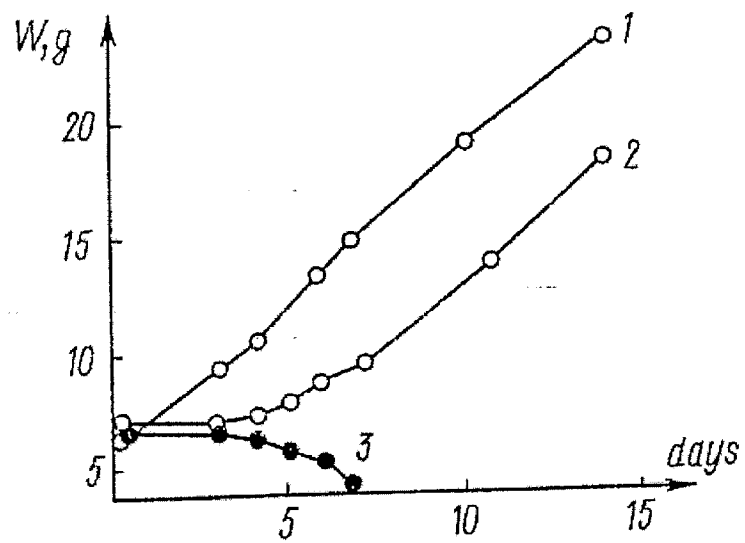


FIG.2

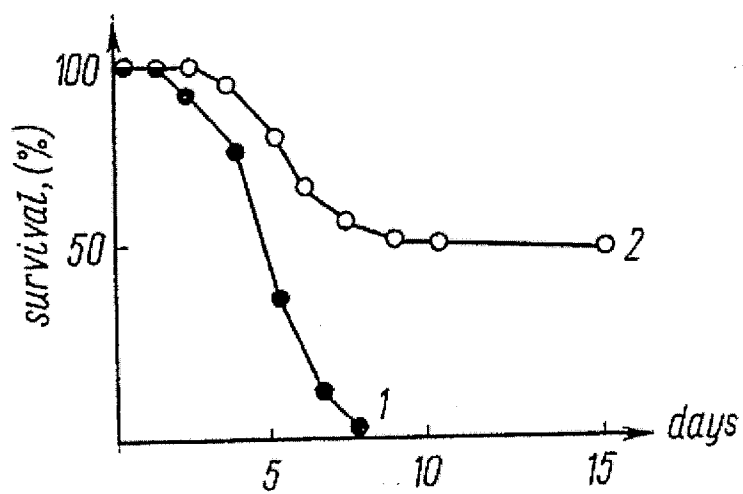
$\frac{2}{3}$ 

FIG. 3a

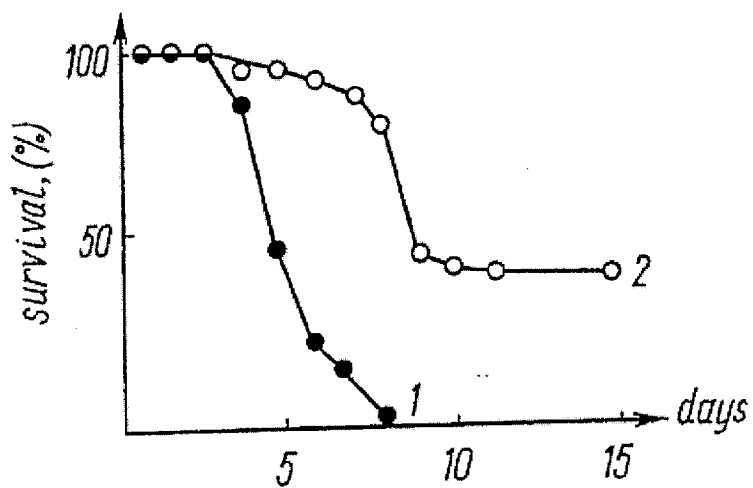


FIG. 3b

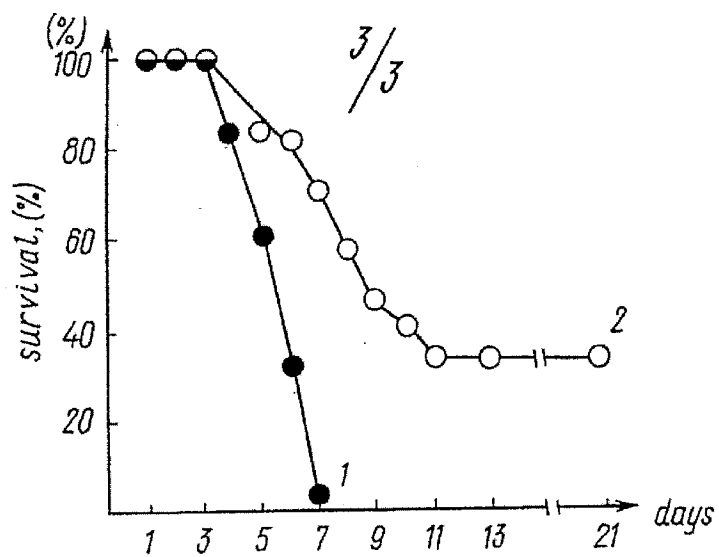


FIG. 3c

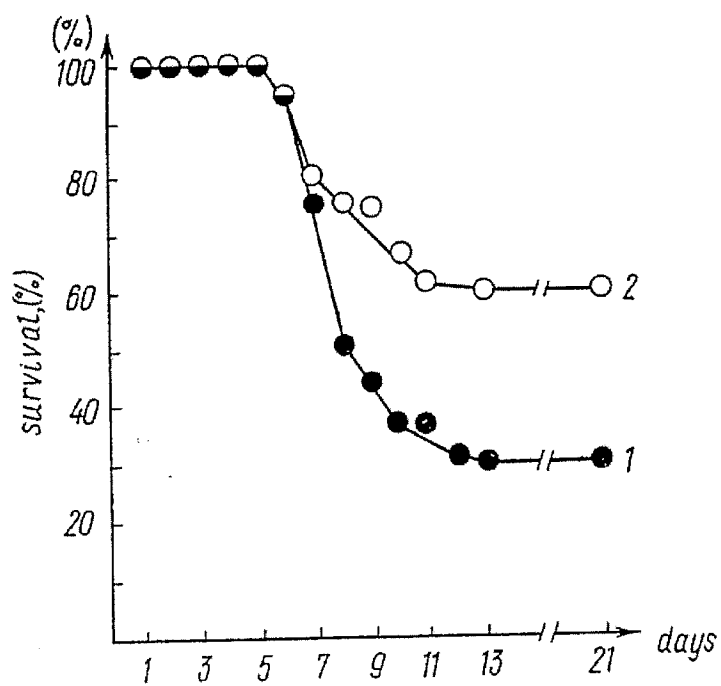


FIG. 3d

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int. Cl. 5 A61K 9/12, 37/64		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
Int. Cl. 5 A61K 9/12, 9/72, 37/02, 37/64, 47/00, 47/44		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB, A, 2 082 457 (ELI LILLY AND COMPANY), 10 March 1982 (10.03.82), the claims	1-11
A	EP, A1, 0 371 706 (NIHON CHEMICAL RESEARCH KABUSHIKI KAISHA), 6 June 1990 (06.06.90)	1-11
A	FR, A1, 2 646 354 (SANOFI), 2 November 1990 (02.11.90) the claims	1,9
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
13 October 1992 (13.10.92)		21 October 1992 (21.10.92)
Name and mailing address of the ISA/ ISA / RU		Authorized officer
Facsimile No.		Telephone No.